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Cancer Stem Cells in Drug Resistance and Drug Screening: Can We Exploit the Cancer Stem Cell Paradigm in Search for New Antitumor Agents?

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1. Introduction

It is now evident that despite an enormous progress in our understanding of molecular mechanisms and processes which operate in tumor cells, this knowledge does not directly translate into more efficient treatment and cure of cancer patients. At the origin of the inefficiency of cancer treatment is inherent or therapy-induced resistance of tumor cells to therapeutic agents. Many different mechanisms of drug resistance have been described and characterized, including elevated expression of membrane drug transporters, changed drug activation and/or detoxification, more efficient repair of drug-induced lesions and non-functional cell death pathways. Another reason for an inefficiency of currently available cancer treatment modalities can be related to cellular heterogeneity of tumors and targeting by anticancer drugs only some tumor cells in the population, which are more sensitive to applied therapeutic agents. In this situation, anticancer treatment can lead to the selection of drug resistant tumor cells and cytostatic rather than cytotoxic effect. The origin of these more resistant cells in tumor cell population was classically perceived as a result of spontaneous or therapy-induced gene mutations, making surviving tumor cells less sensitive to anticancer agents. According to the alternative hypothesis, heterogeneity of tumor cell population, also in its response to drug treatment, can result from a clonal expansion of rare malignant stem cells which may differentiate and produce tumors.

Cancer was proposed to originate from stem cells more than 150 years ago (see Wicha et al., 2006 and references therein) and this idea re-appeared in the early sixties of the last century, first for leukemias (Bruce & van der Gaag, 1963) and later for epithelial tumors (Hamburger & Salmon, 1977). About the same time, Pierce and Wallace provided experimental evidence for the existence of cellular hierarchy in tumors, where malignant undifferentiated cells give rise to benign well-differentiated cells (Pierce & Wallace, 1971). A few years later, Potter proposed a new model of oncogenic transformation according to which tumor cells resulted from blocked differentiation of their progenitors (Potter, 1978). Collectively, a new paradigm of cancer origin was established in which malignant stem cells with de-regulated self-renewal and differentiation mechanisms are responsible for tumor initiation and growth.

Recent explosion of reports providing experimental data that confirm this hypothesis is undoubtedly associated with a growing knowledge about normal stem cells and their potential practical applications in regenerative medicine.

It is known for years that embryonic cells may spontaneously form teratocarcinomas when transplanted into mice. Secondly, when adult differentiated cells are induced by oncogenes and transcription factors to trans-differentiate into pluripotent cells (iPSCs) with stem-like features, they form tumors in experimental animal models with a relatively high frequency (for recent review see Yamanaka & Blau, 2010). Accumulating evidence show that there are remarkable similarities between the reprogramming processes and oncogenic transformation of adult somatic cells and similar factors regulate both pluripotency and tumorigenicity. For example, it was shown that cell reprogramming is regulated by p53, p16 (INK4a) and p21 (Banito et al., 2009) similarly to tumors. Both reprogramming and oncogenic transformation require specific combinations of collaborating genes, that can produce a less differentiated cell able to proliferate and self-renew indefinitely. All four factors, which were initially shown to reprogram somatic cells to iPS cells, are overexpressed in at least some types of tumor, and two of them – *c-myc* and *Klf4* – are established oncogenes. Similarly, reprogramming is less efficient in cells which are close to senescence suggesting that, similarly as in tumorigenicity, cellular senescence protects cells from induced pluripotency (Banito et al., 2009). Together, one can conclude that studies on reprogramming of adult somatic cells into iPS cells provide probably the best experimental evidence that the idea of abnormal stem cells as the origin of tumors may actually be true. It seems that today it is more and more important to delineate similarities and differences between tumor cells and iPS/stem cells as it may provide insights into cancer origin and potentially give new clues for anticancer drug screening and therapy (for recent review see Tilkorn et al., 2010).

There are many controversies related to the cancer stem cell paradigm and its importance for anticancer therapy, some of them will be presented in the following sections of this chapter. To begin with, there is a problem with terminology since the term ‘cancer stem cells’ (CSC), that is used quite commonly, is somewhat confusing as it relates cancer cells to true stem cells, and this is still hypothetical. Introduction of the name ‘stemloids’ by Blagosklonny is less ambiguous since it implies some similarity to stem cells, however, pointing to important differences (Blagosklonny, 2005). Another term that is in use, ‘tumor-initiating cells’, is also confusing since it suggests that these are the cells that have initiated the tumor *in vivo*. The most relevant is probably the term ‘tumor-propagating cells’, introduced by Kelly et al (Kelly et al., 2007) and Hong et al (Hong et al., 2008), that points to the ability of tumor cells to propagate both *in vitro* and *in vivo*. In this review, we will use the term cancer stem cells (CSCs) as it still is the most popular in the current literature.

It is still not clear as to whether the cancer stem cell paradigm can be applied to all human tumors or it is restricted to leukemias and several types of solid tumors. Moreover, cancer stem cells have been shown to be resistant to anticancer agents but molecular mechanisms, which are responsible for drug resistance phenotype of these cells, are far from being fully characterized. Finally, it is not at all clear how to include the knowledge about cancer stem cells, that we accumulated so far, into new methodologies and assays used in drug screening, both in cytotoxicity measurements *in vitro* and antitumor assays in animal models. Without these practical tools, it will be impossible to screen for new drugs and drug combinations that will allow us to eradicate CSCs and in consequence tumors.

2. Identification and quantitation of cancer stem cells in human tumors

The existence of rare stem-like cells is being experimentally confirmed in a growing number of different tumor types (for review see Reya et al., 2001; Pardal et al., 2003), including myeloid leukemia, breast carcinomas, glioblastoma, melanoma, lung and colon carcinomas (Quintana et al., 2008; Li et al., 2008; Meng et al., 2009; Yeung et al., 2010). One of the most astonishing discoveries concerning CSCs was identification of cancer cells with stem-like properties in cell populations from established tumor cell lines maintained *in vitro* (Yeung et al., 2010). This discovery has important implications as it opens a possibility to use tumor cells cultivated *in vitro* in drug screening and find new compounds which are able to kill CSCs (see section 5 of this chapter).

One of the controversial issues in the field is whether all known tumor types are heterogeneous and consist of a small fraction of CSCs that is able to produce tumors *in vivo* and differentiate to non-CSC cells. It has been shown that human colon carcinoma HCT-116 cells do not contain a hierarchy of tumor cells as concerns production of tumors in nude mice (Kai et al., 2009; Diettfeld et al., 2010). Another example is glioblastoma C6 cells where the majority of cell population formed tumors *in vivo* although these cells have only 0.4% side population (SP) cells (Zheng et al., 2007). Does it mean that in these tumor cell populations all cells have features of CSC? Is it a typical situation or rare examples between tumors of epithelial origin? Similarly, the fraction of CSCs in different human tumors is very divergent. This is at least partially related to the fact that estimations of the number of CSCs are based on several different methodologies (discussed below). Moreover, all of them assume that the CSC fraction is homogenous and can be clearly distinguished from non-CSC cells, and this has never been firmly established. In contrast, there are reasons to believe that CSC cells, with the capacity for long-term self-renewal, constitute an identifiable subpopulation of tumor cells but there is a hierarchy of stem-like cancer cells, as has been recently shown for glioblastomas (Chen et al., 2010).

The most popular strategy to identify CSCs uses specific cell surface markers, such as CD34, CD44, CD117, CD133, integrin $\alpha 2\beta 1$, ESA (epithelial specific antigen) and others or their combinations. A different approach is to mark side population (SP) cells based on the exclusion of Hoechst 33342 dye, as the SP fraction is postulated to be enriched in CSCs (Hirschmann-Jax et al., 2004; Patrawala et al., 2005). However, there are reports showing that expression of membrane markers can not reliably distinguish between CSCs and non-CSCs and cells expressing specific stem cell markers can be as tumorigenic in nude mice as tumor cells which are devoid of specific stem cell markers. This has been particularly well documented for a commonly used stem cell marker CD133 in glioblastomas (Beier et al., 2007; Prestengarden et al., 2010; Chen et al., 2010) but also in colon carcinoma HCT-116 cells (Dittfeld et al., 2010).

CSCs are also frequently quantitated based on the number of cells which are tumorigenic, when transplanted into immunocompromized mice. Although most researchers of the cancer stem cell community consider the latter method as the most reliable, there are also reports suggesting that one has to be very cautious when interpreting results of these tests. It seems, for example, that one may greatly underestimate the frequency of tumorigenic cells in tumor cell population as it depends on the animal model used. Recent studies showed that the detection of tumorigenic melanoma cells injected into mice can be increased by several orders of magnitude if more highly immunocompromised NOD/SCID interleukin-2 receptor gamma chain null (Il2rg(-/-)) mice are used in the modified xenotransplantation

assay and tumor cells are injected in Matrigel (Quintana et al., 2008). In these conditions, the estimated fraction of melanoma CSCs from cancer patients increased to about 25%, suggesting that these cells are much more common, at least in some human tumors. Therefore, it is possible that estimations of CSC number by tumorigenicity assay may be biased by the way this assay is performed i.e. animal model used (nude mice vs. SCID mice, or SCID mice with no residual immunity), the number of tumor cells injected into animals, etc.

Some authors postulate that human tumor cells may also differ in their ability to produce tumors in mice and this factor may also greatly influence estimations of CSCs fraction (Baker, 2008). If this is true, one may argue whether the xenotransplantation assay really detects a rare subset of cells that can propagate tumors (i.e. which are tumorigenic) or a rare subset of human tumor cells that can establish themselves in mice. It should also be noted that staining by Hoechst 33342 may be toxic for CSCs as shown for C6 glioblastoma cells and cell sorting by flow cytometry, that is based on Hoechst 33342 stainability, may considerably lower tumorigenicity and, in effect, hamper accurate estimation of CSC fractions (Shen et al., 2008).

The origin of CSCs both in *in vivo* tumor models and in tumor cell lines maintained in *in vitro* culture is another controversial issue. According to the standard CSC hypothesis, the initial CSC originates from normal stem cells or by re-programming more differentiated progenitor cells by oncogenic insults or both. Propagation of these initial CSCs is based on asymmetrical cell division and production of other CSCs and non-CSCs. However, it should be noted that other mechanisms have been proposed by which CSCs may be formed. These include epithelial to mesenchymal transition induced in non-CSC cells from mammary carcinoma that has recently been shown to be involved in CSC formation (Mani et al., 2008; Morel et al., 2008; Santisteban et al., 2009). Some other phenomena related to anticancer therapy may also be implicated in CSC formation. De-differentiation of non-CSC cells into CSCs may be favored by stress-induced factors released after drug treatment. Moreover, many anticancer drugs induce growth arrest in G2 and M phases that frequently leads to polyploidization. Drug-induced polyploidy usually leads to cells death by mitotic catastrophe (for review see Vakifahmetoglu et al., 2008) but in some situations the process of de-polyploidization may occur and result in CSC production (Erenpreisa & Craig, 2010; Salmina et al., 2010). Polyploidy and consequently cancer stem cells may be also produced by cell-cell fusion (Rizyj et al., 2006; Dittmar et al., 2009). These effects can also explain the effect of the so-called 'oncogenic resistance', the phenomenon frequently observed in the clinical situation, where after treatment with therapeutic agents tumor cells are both more malignant and resistant to anticancer therapy, compared to untreated tumor cells. It seems that 'oncogenic resistance' can not be attributed to resistance phenotype of CSCs (discussed in Dittmar et al., 2009). One possibility is that DNA damage induced by anticancer treatment may lead to gross genomic re-arrangements (both genetic and epigenetic) that result not only in acquiring by tumor cells the stem-like phenotype but also in drug resistance. Changes in tumor cell genome may occur during de-polyploidization of polyploid cells, followed by abnormal mitotic divisions that lead to reduction of cellular DNA content. CSCs can also be generated by cell-cell fusion that is also stimulated by anticancer treatment (Dittmar et al., 2009). According to this scenario, fusion between e. g. CSC and non-CSC cells may result in tumor cells with stem properties. This approach has been used to produce pluripotent cells from adult somatic cells (discussed in Pralong et al., 2006).

3. Properties of stem cells and CSCs: similarities and differences

One of the issues concerning CSCs that needs clarification is which properties are shared between normal stem cells and cancer stem cells. This is particularly important for potential therapies directed toward CSCs that should spare normal stem cells. CSCs are defined to have three features: i) represent a minor population of a tumor (typically 0.1-2% of all cells but may be as high as 25% - see previous section); ii) have the property of self-renewal; iii) are the only cells within the tumor which are capable of immortal growth and production of the tumor *in vivo*. It follows that tumor cell population is composed of relatively rare CSCs and 'committed' or 'differentiated' non-CSC tumor cells with possibly limited life span. Important properties of normal stem cells include self-renewal, as well as pluripotency i. e. the ability of stem cells to differentiate to many functionally distinct cell types. In addition to that, stem cells are characterized by their very limited proliferation potential, as stem cells divide only occasionally and in response to very specific intra- and extracellular signals.

Some of these stem cell features cannot directly be applied to CSCs. Most of the available literature data show that CSCs proliferate quite rapidly, although doubling times are frequently much slower compared to the non-stem tumor population (Ropolo et al., 2009; Ishimoto et al., 2010). An extreme case can be leukemia stem cells which are commonly dormant but can be induced to proliferate by specific cytokines or anticancer agents such as arsenic oxide (Essers & Trumpp, 2010; Thomas & Cannas 2010). Secondly, one of the features of stem cells is their ability to produce more differentiated cell progeny. Accordingly, cells of many tumor types are able to differentiate reversibly or irreversibly into different cell types. For example, irreversible differentiation of myeloid leukemia HL-60 cells into monocytes or granulocytes is induced by sodium butyrate, forskolin and hexamethylene bisacetamide (Breitman et al., 1990) or re-activation of wild-type p53 (Soddu et al., 1994). Similarly, colon carcinoma HT-29 cells may be reversibly differentiated into enterocytes or mucin-producing lineages (Chakrabarty et al., 1992; Choi et al., 2000; Demers et al., 2009), and another colon carcinoma SW1222 cells differentiate into enterocytes, enteroendocrine and goblet cells (Yeung et al., 2010). Yet another classical example is breast carcinomas such as MCF-7 cells which may also be induced to differentiate by sodium butyrate and forskolin (Wasserman et al., 1987; Guilbaud et al., 1990). Conversely, nuclear transfer studies showed that the phenotype of at least some cancer cells (as shown for melanomas) can be reversed to a pluripotent state that allows apparently normal differentiation (Hochedlinger et al., 2004). Unfortunately, it is not known whether tumor cell nuclei used for nuclear transfer were from CSCs or non-CSC cells. Another example of tumor cell plasticity related to cell differentiation is the work of Kulesa et al where metastatic phenotype of melanoma cells was shown to be reversed by embryonic milieu (Kulesa et al., 2006). These data point to the important role of tumor microenvironment for the maintenance of tumor cell phenotype.

As mentioned above, there are also tumor cells e. g. HCT-116 where there is apparently no hierarchy of cells differing in tumorigenicity (Kai et al., 2009) and which have little or no capacity to differentiate, therefore, in these tumors majority (if not all) cells can be considered as CSC. This suggests the existence at least two types of tumors: these with CSC sub-population which are able to differentiate and those, which contain only cells with CSC features and no or limited differentiation capacity. An intriguing question remains whether in cells like HCT-116, differentiation of CSC cells is irreversibly blocked or it may still be

activated in stress conditions imposed for example by a drug treatment. This is important given that in tumor cell populations, where no hierarchy is observed, drug response and sensitivity should be more homogenous so *in vitro* testing for cytotoxic activity gives more reliable results as for drug concentrations required to eradicate all tumor cells. In contrast, if tumors contain populations of cells, with various in their differentiation status, cytotoxic action of antitumor drugs may also be heterogeneous. In that case, drug screening with mixed tumor cell populations can provide information about overall sensitivity of tumor cells only if the cytotoxic effect is determined in such a way that distinguishes killing or not of both CSCs and more differentiated non-CSC cells.

Finally, according to a classical stem cell hypothesis differentiated non-CSC cells have a limited life span. However, very little is known about molecular mechanisms which can explain the potential limited proliferation capacity of non-stem tumor cells and the ultimate fate of non-stem tumor cells. This may be related to the fact that relatively little research activity has been devoted to non-CSC tumor cells.

It is well known that in cell culture there is always a small fraction of apoptotic cells but where the fraction of dead cells comes from, whatever low it is, is not clear. Is this slow but progressive shedding of differentiated tumor cells? It is possible that these dying 'mature' tumor cells reached the survival limit due to the number of cells divisions. Another interesting point is whether there is a 'Hayflick-like limit' for differentiated tumor cells, if it at all exists? If positive, what is the mechanism of survival limit of tumor cells if it most probably does not depend on telomere-length maintenance? Are CSCs immortal cells?

It should be remembered that molecular mechanism(s) of cell senescence-like process is still active in tumor cells as it can be induced by several different stimuli such as expression of oncogenes, stress conditions or DNA damage (Roninson, 2003). This process resembles replicative senescence but is usually not associated with telomere shortening. Surprisingly, relatively recent studies have shown that human hepatocarcinomas and immortal breast carcinoma cells both *in vitro* and in animal *in vivo* models produce spontaneously senescent progeny (Ozturk et al., 2006). It is not clear which type of cells (i.e. CSCs or non-CSCs) had limited proliferation potential and were able to undergo cellular senescence. Analysis of cell clones generated from single cells of breast carcinoma tumor cell lines *in vitro* showed that within 12 different cells lines tested there were two groups. One group (5 cell lines) produced senescent cells (positive for SA- β -Gal/negative for BrdU incorporation) with high frequency (5-40%) whereas the other group produced less than 5% of senescent cells (Mumcuoglu et al., 2010). Based on these features, the authors classified all breast cell lines studied as senescent cell progenitor (SCP) and immortal cell progenitor (ICP) cell subtypes. Interestingly, ICP cells were much more tumorigenic in immunodeficient mice compared to SCP cell lines. Even more importantly, more tumorigenic ICP cells were deficient in their ability to generate more differentiated progeny, pointing to the fundamental difference between these cell subtypes. It would be extremely interesting to find out whether these two types of cells, with different abilities to produce differentiated progeny and ability to undergo senescence-associated growth arrest, are also present in other tumor types. Equally important would be to establish whether these two types of cell clones correspond to CSC and non-CSC cells.

The presence of senescent cells in tumor cell population have been confirmed by others for prostate, head and neck squamous cell and breast carcinomas (Locke et al., 2005; Li et al., 2008). Molecular mechanism of spontaneous senescence induced in tumor cells was associated with repression of hTERT expression, that led to telomere shortening (Ozturk et

al., 2006), therefore, it followed a classical replicative senescence program. These results provide an experimental evidence for the reversibility of cancer cell immortality by repression of telomerase expression. This is probably not surprising considering the fact that expression of telomerase is switched on in most tumor cells and is regulated by a number of different genes, including *SIP1*, *hSIR2*, *c-myc*, *Mad1*, *Menin*, *Rak*, and *Brit1* as well as TGF- β and SMAD pathway (Wang et al., 1998; Verschueren et al., 1999; Lin & Elledge, 2003). Therefore, the epigenetic mechanism responsible for upregulation of hTERT expression and telomerase activity in a majority of tumor cells is potentially reversible and can be turned off again.

4. Resistance of CSCs to anticancer treatment: possible molecular mechanisms

It is well established that a fraction of cells in a tumor frequently survives anticancer treatment when exposed to radiation and cytotoxic drugs. This drug resistant subpopulation of tumor cells may constitute of CSCs, and in this way, these cells may be responsible for the failure of most, if not all, anticancer therapies, as these cells are postulated to be inherently resistant to anticancer agents. Based on that, a new therapeutic strategy has been proposed in which drugs should specifically target CSCs, and this will allow us to eradicate tumors. However, finding of these CSC-specific agents is only possible if we characterize possible mechanisms responsible for resistance of CSCs to anticancer therapy (summarized in Figure 1). Another important question is whether CSCs are resistant to all therapeutic agents or there are drug-specific resistance phenotypes, associated with changes in the functioning of defined intracellular pathways in these cells. Moreover, drug resistance of CSCs may involve several mechanisms and results from changes in different intracellular pathways. It is also not clear if molecular mechanisms responsible for CSC therapeutic resistance are shared across different tumor types.

The most straightforward mechanism that can be responsible for lower activity of anticancer drugs toward CSCs is overexpression of ABC transporters. One of the methods for CSC determination is based on lower stainability of CSC-containing fraction, the so-called SP cells, to fluorescent dye Hoechst 33342 (discussed in section 2 of this chapter). Low fluorescence of SP cells after Hoechst 33342 staining is attributed to overexpression of ABC pumps by CSCs, frequently ABCG2. Since many antitumor drugs are substrates for ABC membrane transporters, this can lead to typical multidrug resistance phenotype of CSCs. It should be noted, however, that Hoechst-based assay for SP fraction may give misleading results. It has been shown that ABCC1-overexpressing cells HL-60/Adr (Marsch et al., 1986) contain more than 90% of SP cells (Patrawala et al., 2005) that is not necessarily associated with increased CSC content.

Up-regulation of ABC transporters is a typical feature of both normal stem cells and CSCs (Patrawala et al., 2005; Nakai et al., 2009; Yamamoto et al., 2009; Angelastro & Lamé, 2010; Jin et al., 2010). However, this can not be a general phenomenon as our results obtained for non-small cell lung carcinoma A549 cells showed that CSCs cells isolated after treatment with anticancer drugs (dexrazoxane, amsacrine) did not overexpress ABC transporters and had unchanged drug sensitivity (Sabisz & Skladanowski, 2009).

It is interesting that overexpression of ABC transporters in stem cells and CSCs correlates with the level of several stem cell markers such as CD133, nestin, CD117 (c-kit) (Yamamoto et al., 2009; Adhikari et al., 2010) or Notch-1 and Nanog (Patrawala et al., 2005; Bourguignon

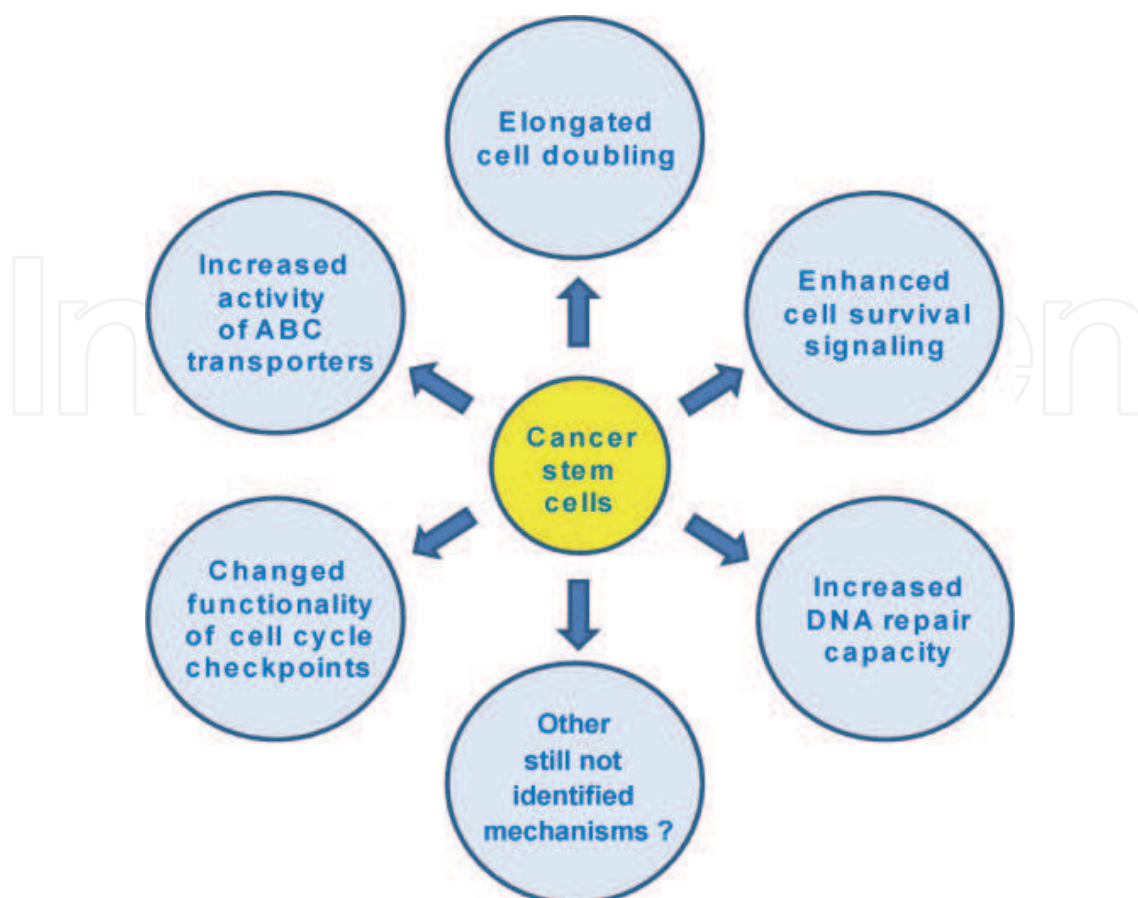


Fig. 1. The proposed mechanisms responsible for drug resistance phenotype of cancer stem cells.

et al., 2008). Inherent overexpression of ABC pumps in these cells is dependent on stem factors such as Oct4, that is present both in embryonic stem cells and CSCs (Wang et al., 2010) and may be induced by anticancer treatment (Nakai et al., 2009). Similarly, interaction between stem cell related transcription regulators STAT1/3 and Nanog leads to activation of STAT1/3 and increased expression of several genes, including ABCB1 transporter (Bourguignon et al., 2008). Given the fact that CSCs frequently overexpress ABC transporters, it is interesting to note that salinomycin, that has been shown to selectively kill mammary carcinoma CSCs (Mani et al., 2008) is the inhibitor of ABCB1 pump (Ricconi et al., 2010).

Alternative drug resistance mechanism of CSCs may be associated with lower proliferation potential of these cells compared to 'committed' non-CSC cells. In this situation, anticancer treatment that targets actively proliferating cells, such as DNA damaging agents, mitotic spindle poisons or antimetabolites, are less effective in killing CSCs than mature 'differentiated' cancer cells. Although this issue has not been systematically studied, several groups reported that CSCs isolated from gastric carcinomas and glioblastomas have either increased (Beier et al., 2008) or elongated (Ropolo M et al., 2009; Ishimoto et al., 2010; Thomas & Cannas, 2010) doubling time compared to a bulk tumor cell population or non-CSC cells. Surprisingly, changes in doubling time are not always associated with differences in the distribution between cell cycle phases (Ropolo M et al., 2009). As discussed above, in hematological malignancies, leukemia stem cells usually do not proliferate.

It is not clear whether longer doubling times are characteristic for CSCs in all types of tumors and if they result from fundamental differences in cell cycle regulation between CSCs and differentiated tumor cells. It should be noted that determination of a doubling time for CSCs and non-CSCs was performed in the artificial situation where these two cell populations grow separately, and this may influence their growth rate. The existence of a possible interaction between CSCs and non-CSC cells and other cells from tumor microenvironment can be concluded based on results obtained in colon carcinoma, glioma and leukemia models (Evers et al., 2010; Raaijmakers et al., 2010; Saito et al., 2010; Vermeulen et al., 2010). Perturbations of mechanism(s) of cross-regulation of cell growth, which potentially exist between CSCs and non-CSCs, may also influence drug sensitivity (see next paragraph).

Another mechanism that can be proposed to explain drug resistance phenotype of CSCs is related to differences between CSCs and non-CSCs in the functionality of cell cycle checkpoints and enhanced repair of drug-induced damage. However, this is controversial as there are contradictory data in the available literature concerning this issue. Defective intra-S checkpoint but intact G2 checkpoints were documented in glioblastoma CD133-positive stem-like cells isolated from patient tumor samples. These cells showed increased sensitivity to irradiation with respect to the standard glioblastoma model, established glioma cell lines (McCord et al., 2009a). Interestingly, when radiosensitivities of CD133-positive and negative cells from glioma cell lines were compared, CD133+ stem-like cells showed radioresistance (McCord et al., 2009a). Other studies have shown that glioma CSCs as well as epithelial cells with stem-like properties, preferentially activate DNA damage response and cell cycle checkpoints after treatment with ionizing irradiation both *in vivo* and *in vitro* and this can be related to their radioresistance (Bao et al., 2006; Facchino et al., 2010; Harper et al., 2010). Molecular mechanism that was responsible for lower sensitivity to irradiation of glioma CSCs involved increased activity of two intra-S and G2 checkpoint kinases, Chk1 and Chk2. Inhibition of these kinases by a selective chemical inhibitor debromohymenialdisine sensitized CSC cells to irradiation (Bao et al., 2006; Harper et al., 2010). Interestingly, increased Chk1 and Chk2 activity was also shown in untreated glioma CSCs (Ropolo et al., 2009), suggesting that enhanced basal activation of checkpoint kinases in CD133+ cells may determine their cell cycle delay and contribute to their radioresistance by allowing more time for DNA repair of damages.

The role of DNA repair in radio- and chemoresistance of CSCs is less clear. In one report, no differences in DNA base excision or single-strand break repair nor in resolution of γ -H2AX nuclear foci were found in radioresistant CD133+ CSCs compared with CD133- glioma cells (Ropolo et al., 2009). However, earlier study with glioma tumor cells treated with temozolomide showed increased rather than decreased sensitivity of CD133-positive CSCs (Beier et al., 2008). The drug produced essentially no cell death but a prominent growth inhibitory effect was observed specifically for glioma stem cells. In contrast, temozolomide did not inhibit the growth of progenitor and differentiated cells derived from CSC but showed a selective growth inhibitory effect toward glioma CSCs. Temozolomide is the most commonly used chemotherapeutic agent in the treatment of glioblastomas and induces DNA adducts which are repaired by the DNA repair protein O⁶-methylguanine-DNA-methyltransferase (MGMT). MGMT is expressed only in a subgroup of glioblastomas since its promoter is frequently methylated in this type of tumor cells (Hegi et al., 2005). Accordingly, temozolomide concentrations required to deplete glioma CD133+ CSCs was

substantially higher (about 10-fold) in tumor cells expressing MGMT. However, combination of the drug with the MGMT inhibitor 6-buthylguanine sensitized stem cell-like glioma cells with high MGMT expression to the deleterious effects of temozolomide. Finally, radio- and chemoresistance of CSCs may be associated with the interplay between DNA damage response induced by anticancer treatment and regulated by the ATM/ATR pathway as well as survival signaling mediated by PI3K/Akt pathway (for recent review see Skladanowski et al., 2009). It was postulated that regulation of DNA damage response induced by irradiation in CSCs follows the classical ATM-dependent mechanism (Facchino et al., 2010; Golding et al., 2009). Furthermore, a specific ATM inhibitor KU-60019 reduced basal activation of Akt by downregulation of its Ser-473 phosphorylation and this led to reduced glioma cell migration and invasion (Golding et al., 2009). In this way, inhibition of DNA damage response by KU-60019 is associated with downregulation of pro-survival signaling mediated by Akt and sensitizes glioma cells to irradiation. The important role of Akt pathway in stemness and invasion but also in response to cancer treatment has been confirmed by others in gliomas (Molina et al., 2010; Wang et al., 2010), but also in lung, colon and mammary carcinomas (Sabisz & Skladanowski, 2009; Wang et al., 2010; Zhang et al., 2010). Interestingly, there may be a cross-talk between at least some stem cell markers, such as CD44, and PI3 kinase/Akt-related survival pathways, can also lead to chemotherapeutic drug resistance, as shown in breast tumor cells (Miletti-González et al., 2005). Collectively, these results raise important therapeutic implications for a concurrent combination of DNA damaging drugs and inhibitors of Akt pathway to target CSCs (Mueller et al., 2009; Sabisz & Skladanowski, 2009; Zhang et al., 2010).

5. Perspectives: can we exploit the CSC paradigm in drug screening?

There is accumulating experimental evidence that CSCs are resistant to standard anticancer therapies. This suggests that screening procedures, in which cytotoxic and antitumor effect induced by antitumor agents is evaluated, should be modified in such a way to select drugs or drug combinations which are able to target CSCs. Current *in vitro* drug screening systems, with the most widely known the NCI 60 cell line model, are based on a relatively short-term drug treatment and continuous drug exposure of tumor cells. The cytotoxic effect is evaluated using different tests which are sensitive to drug-induced changes of tumor cell number and/or viability, using typically the MTT assay (reduction of the MTT stain to formazan), chemiluminescence-based measurements of intracellular ATP content.

Several different experimental approaches were proposed for screening of CSC-specific antitumor agents (see Figure 2). In one of them the standard cytotoxicity assays are performed using CSC populations which are sorted based on the expression of membrane stem markers by e.g. flow cytometry or immunomagnetic cell sorting. The cytotoxic effect toward CSCs is then compared with that induced in non-CSCs. A simpler variant of this methodology is to sort the SP cells as a fraction of cells with high content of CSCs. This method can also be applied to evaluate antitumor effect of drugs *in vivo* using animal models.

Another way of enrichment of tumor cell populations with CSCs is the generation of cell spheres at conditions when the attachment of tumor cells to the substratum is prevented. This could be realized in several different ways, the most typical involves low attachment substrata where cell dishes are covered both by natural (e. g. agar) or synthetic polymers. In a more technically sophisticated approach, magnetic levitation is used where tumor cells are cultivated in hydrogels on magnetic iron oxide nanoparticles, with magnetic-controlled

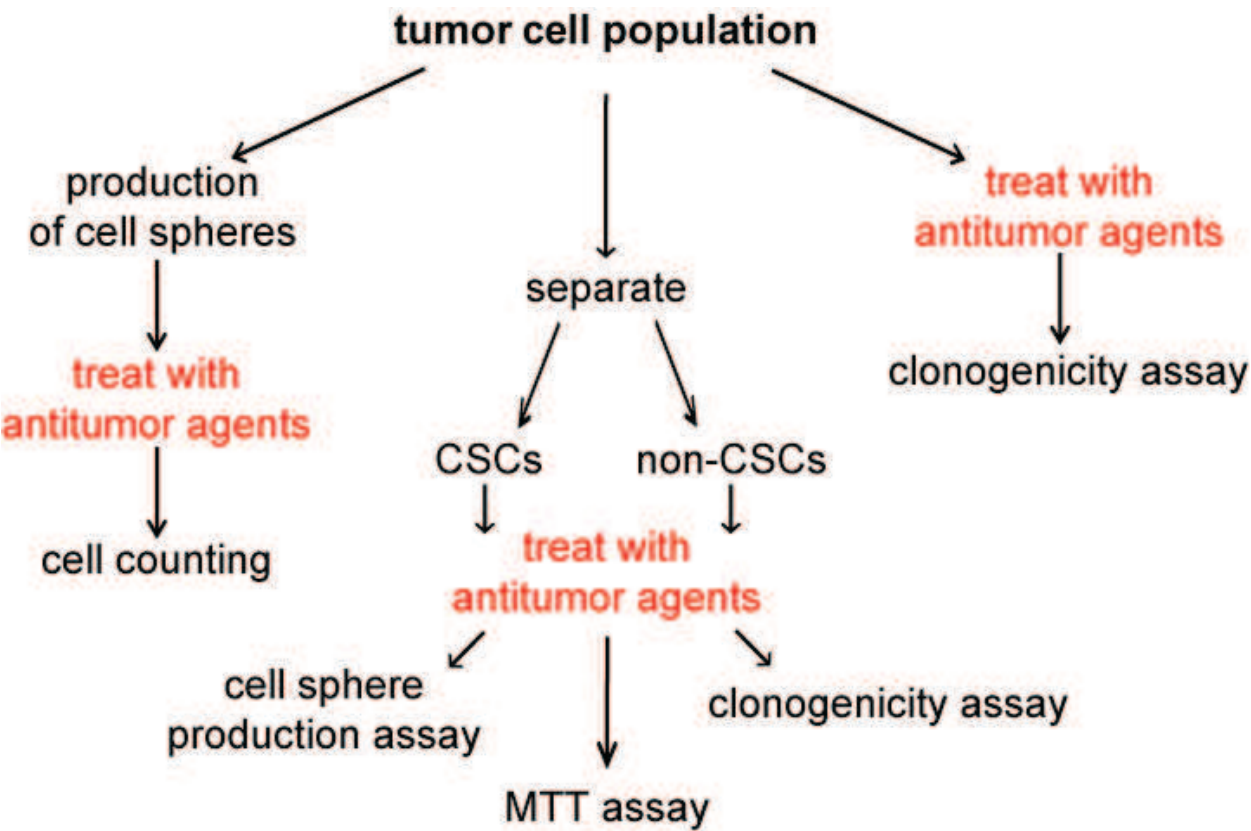


Fig. 2. Currently available strategies which can be used to screen for drugs or drug combinations with selective activity toward cancer stem cells.

levitation system (Souza et al 2010). Interestingly, magnetically levitated human glioblastoma cells showed similar protein expression profiles to those observed in human tumor xenografts (Souza et al 2010). The sphere formation models were used to determine the cytotoxic activity of antitumor drugs of different tumor types, including mammary carcinomas (mammospheres), gliomas (neurospheres) and lung carcinomas (Setoguchi et al., 2004; Patrawala et al., 2005; Levina et al., 2008; Bertolini et al., 2009). It is worth mentioning that the seminal work of Robert Kerbel and his co-workers on drug resistance of tumor cells, associated with what was at this time called the ‘social effect’ (Kobayashi et al 1993), can be related today to the CSC phenomenon.

Yet another possibility is to treat bulk tumor populations with different antitumor agents and the fraction of tumor cells, which are able to proliferate after drug treatment, is subsequently estimated based on the colony formation ability or production of spheres on low adherent plates by drug-treated tumor cells. The latter approach was successfully applied for lung, breast and ovarian carcinoma cells (Levina et al., 2008, Sabisz & Skladanowski, 2009). In addition, to confirm that cells surviving after drug treatment are truly CSCs, cells may be also analysed for the expression of stem markers.

The features of CSCs that were presented in this chapter suggest that there are two groups of potential problems related to the described above screening assays and possibly other screening methods aimed at the selection of anti-CSC drugs. These problems may potentially makes it very difficult to apply the CSC paradigm in search for new therapeutic strategies to kill CSCs. First group is associated with problems which we call ‘technical’ such as cell systems used and estimation of the cytotoxic effect of potential drugs toward CSCs.

Classical cytotoxicity assays such as MTT or ATP-based chemoluminescence tests can not be applied in sphere formation systems. In multicellular spheroids, there is a problem with the penetration of biochemical stains used in these assays and mitochondrial activity of cells present inside spheroids is greatly reduced, that underestimates the number of viable cells in spheroids. On the other hand, colony formation assays are difficult to be performed in high throughput systems where drug screening is highly automated and a very small number of cells is used. In this situation, results may be irreproducible and experimental errors may be exceedingly high. As for cell systems used to screen for anti-CSC drugs, it should be borne in mind that cell spheres are not always enriched in CSCs (Gasparini et al., 2010). In addition, there are no generally accepted stem markers of CSC that makes it very difficult to reliably separate these cells from non-CSCs very difficult (discussed in section 2 of this chapter). In addition, expression of some stem markers may be functionally inter-related and downregulation of marker 1 expression may lead to increased/decreased expression of marker 2. This kind inter-relationship in membrane marker expression has been shown in breast carcinoma cells for $\beta 1$ integrin and EGFR (Wang et al 1998).

Cell growth conditions used to cultivate CSCs to be used in drug screening may also be problematic. Typical growth media containing serum may change cancer stem cell phenotype and their characteristic gene expression profile. It has been shown that maintenance of glioblastoma CSCs in media with defined growth factors such as β -FGF and EGF (stem conditions) preserve more closely stem-like phenotype of these cells but serum (differentiation conditions) induces irreversible cell differentiation (Lee et al 2006). Similarly, oxygen concentration in cell culture may be critical for the preservation of the CSC phenotype. It has been shown that the SP fraction in tumor cell population *in vivo* is increased in hypoxic regions (Das et al 2008). This effect was reproduced with the same cell system by exposure of cultured cells *in vitro* to hypoxia. The authors propose that a highly tumorigenic SP cells migrate to the area of hypoxia that may serve as a niche for the highly tumorigenic fraction of SP cells and can be induced *in vitro*. Moreover, increasing evidence suggest that hypoxia has the potential to inhibit tumor cell differentiation that leads to increased fraction of CSCs in hypoxic regions that results result in accelerated the initiation and growth of tumors (Calabrese et al 2008). An elegant study has shown that growing CD133(+) cells sorted from three GB neurosphere cultures at 7% oxygen reduced their doubling time and increased the self-renewal potential as reflected by clonogenicity (McCord et al., 2009b). Furthermore, at 7% oxygen, the cultures exhibited an enhanced capacity to differentiate along both the glial and neuronal pathways. As compared with 20%, growth at 7% oxygen resulted in an increase in the expression levels of the neural stem cell markers CD133 and nestin as well as the stem cell markers Oct4 and Sox2 (McCord et al., 2009b). Collectively, these reports point to still greatly underestimated role of hypoxia in the maintenance of the CSC properties.

Another group of problems is related to our current understanding of the CSC paradigm. It seems that the most fundamental question is whether a tumor develops from a homogenous but scarce population of CSCs, as the classical hypothesis of cancer stem cells proposes. In this case, it makes very much sense to search for the Achille's heel of such a population and use it as a drug target for selective killing of CSCs. However, an emerging picture is that CSCs do not constitute a homogenous population of cells, with defined molecular markers and cell features, as it is still widely believed. There is also accumulating evidence that the

phenotype of CSCs is not stable and, at least in some situations, is reversible. In addition, differences between CSCs and 'mature' tumor non-CSC cells are frequently only quantitative not qualitative, and result from stochastic rather than deterministic processes. Drug selectivity can be a difficult problem to resolve in anti-CSCs therapies. All the already characterized drug resistance mechanisms, which are active in CSCs and responsible for their drug resistance phenotype, operate in tumor cells in general, others are characteristic for normal stem cells. For all these reasons, selection of new therapeutic approaches that specifically target CSCs is a particularly challenging task. To achieve this goal, it is important to define optimal therapeutic targets in CSC sub-populations as well as to implement the improved drug screening systems.

In our opinion a modern and more effective antitumor therapy should include both CSC and non-CSC drugs, ideally targeting both cell populations with high efficacy. Therapeutic eradication of CSCs by their selective targeting with antitumor drugs may be a very dangerous therapeutic approach for several reasons. First, paradoxically many available literature data suggest that very selective antitumor drugs frequently are not efficacious in the clinical practice. Second, we still do not know how many cell generations are required for a tumor to degenerate, as a result of CSC depletion. It is possible that by targeting only CSCs the remaining non-CSCs may well kill the patient before a tumor disappears. In addition to that, although formally non-CSC tumor cells are more sensitive to anticancer agents, so they should not survive drug treatment, there is still a possibility that drugs can initiate cellular processes leading to trans-differentiation of non-CSC to CSC phenotype (depolyploidization, EMT etc.).

Together, more detailed fundamental knowledge is still required about molecular mechanisms responsible for CSC formation, both inherent and therapy-induced, and CSC phenotype in general. Only after understanding these mechanisms will it be possible to find new anticancer treatment modalities which will be able to kill or arrest CSC growth by inhibiting critical intracellular pathways associated with stemness or CSC differentiation or both.

6. Disclosure statement

The authors are not aware of any biases that might affect the objectivity of this review.

7. References

- Angelastro, J.M.; Lamé MW. (2010). Overexpression of CD133 promotes drug resistance in C6 glioma cells. *Molecular Cancer Research*, 8, 8, 1105-1115.
- Adhikari, A.S.; Agarwal, N.; Wood, B.M.; Porretta, C.; Ruiz, B.; Pochampally, R.R.; Iwakuma, T. (2010). CD117 and Stro-1 identify osteosarcoma tumor-initiating cells associated with metastasis and drug resistance. *Cancer Research*, 70, 11, 4602-4612.
- Baker, M. (2008). Melanoma in mice casts doubt on scarcity of cancer stem cells. *Nature*, 456, 7222, 553.
- Banito, A.; Rashid, S.T.; Acosta, J.C.; Li, S.; Pereira, C.F.; Geti, I.; Pinho, S.; Silva, J.C.; Azuara, V.; Walsh, M.; Vallier, L.; Gil, J. (2009). Senescence impairs successful reprogramming to pluripotent stem cells. *Genes & Development*, 23, 18, 2134-2139.

- Bao, S.; Wu, Q.; McLendon, R.E.; Hao, Y.; Shi, Q.; Hjelmeland, A.B.; Dewhirst, M.W.; Bigner, D.D.; Rich, J.N. (2006). Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature*, 444, 7120, 756-760.
- Beier, D.; Hau, P.; Proescholdt, M.; Lohmeier, A.; Wischhusen, J.; Oefner, P.J.; Aigner, L.; Brawanski, A.; Bogdahn, U.; Beier, C.P. (2007). CD133(+) and CD133(-) glioblastoma-derived cancer stem cells show differential growth characteristics and molecular profiles. *Cancer Research*, 67, 9, 4010-4015.
- Beier, D.; Röhl, S.; Pillai, D.R.; Schwarz, S.; Kunz-Schughart, L.A.; Leukel, P.; Proescholdt, M.; Brawanski, A.; Bogdahn, U.; Trampe-Kieslich, A.; Giebel, B.; Wischhusen, J.; Reifenberger, G.; Hau, P.; Beier, C.P. (2008). Temozolomide preferentially depletes cancer stem cells in glioblastoma. *Cancer Research*, 68, 14, 5706-5715.
- Bertolini, G.; Roz, L.; Perego, P.; Tortoreto, M.; Fontanella, E.; Gatti, L.; Pratesi, G.; Fabbri, A.; Andriani, F.; Tinelli, S.; Roz, E.; Caserini, R.; Lo Vullo, S.; Camerini, T.; Mariani, L.; Delia, D.; Calabrò, E.; Pastorino, U.; Sozzi, G. (2009). Highly tumorigenic lung cancer CD133+ cells display stem-like features and are spared by cisplatin treatment. *Proceedings of the National Academy of Sciences USA*, 106, 38, 16281-16286.
- Bourguignon, L.Y.; Peyrollier, K.; Xia, W.; Gilad, E. (2008). Hyaluronan-CD44 interaction activates stem cell marker Nanog, Stat-3-mediated MDR1 gene expression, and ankyrin-regulated multidrug efflux in breast and ovarian tumor cells. *The Journal of Biological Chemistry*, 283, 25, 17635-17651.
- Blagosklonny, M.V. (2005). Why therapeutic response may not prolong the life of a cancer patient: selection for oncogenic resistance. *Cell Cycle*, 4, 12, 1693-1698.
- Breitman, T.R.; He, R.Y. (1990). Combinations of retinoic acid with either sodium butyrate, dimethyl sulfoxide, or hexamethylene bisacetamide synergistically induce differentiation of the human myeloid leukemia cell line HL60. *Cancer Research*, 50, 19, 6268-6273.
- Bruce, W.R.; Van Der Gaag, H. (1963). A quantitative assay for the number of murine lymphoma cells capable of proliferation in vivo. *Nature*, 199, 4888, 79-80.
- Chakrabarty, S. (1992). Regulation of human colon-carcinoma cell adhesion to extracellular matrix by transforming growth factor beta 1. *International Journal of Cancer*, 50, 6, 968-973.
- Chen, R.; Nishimura, M.C.; Bumbaca, S.M.; Kharbanda, S.; Forrest, W.F.; Kasman, I.M.; Greve, J.M.; Soriano, R.H.; Gilmour, L.L.; Rivers, C.S.; Modrusan, Z.; Nacu, S.; Guerrero, S.; Edgar, K.A.; Wallin, J.J.; Lamszus, K.; Westphal, M.; Heim, S.; James, C.D.; VandenBerg, S.R.; Costello, J.F.; Moorefield, S.; Cowdrey, C.J.; Prados, M.; Phillips, H.S. (2010). A hierarchy of self-renewing tumor-initiating cell types in glioblastoma. *Cancer Cell*, 17, 4, 362-375.
- Choi, S.R.; Cho, M.; Kim, H.R.; Ahn, D.H.; Sleisenger, M.H.; Kim, Y.S. (2000). Biological properties and expression of mucins in 5-fluorouracil resistant HT29 human colon cancer cells. *International Journal of Oncology*, 17, 1, 141-147.
- Das, B.; Tsuchida, R.; Malkin, D.; Koren, G.; Baruchel, S.; Yeger, H. (2008). Hypoxia enhances tumor stemness by increasing the invasive and tumorigenic side population fraction. *Stem Cells*, 26, 7, 1818-1830.
- Demers MJ, Thibodeau S, Noël D, Fujita N, Tsuruo T, Gauthier R, Arguin M, Vachon PH. (2009). Intestinal epithelial cancer cell anoikis resistance: EGFR-mediated sustained

- activation of Src overrides Fak-dependent signaling to MEK/Erk and/or PI3-K/Akt-1. *Journal of Cellular Biochemistry*, 107, 4, 639–654.
- Dittfeld, C.; Dietrich, A.; Peickert, S.; Hering, S.; Baumann, M.; Grade, M.; Ried, T.; Kunz-Schughart, L.A. (2009). CD133 expression is not selective for tumor-initiating or radioresistant cell populations in the CRC cell lines HCT-116. *Radiotherapy & Oncology*, 92, 3, 353–361.
- Dittmar, T.; Nagler, C.; Schwitalla, S.; Reith, G.; Niggemann, B.; Zänker, K.S. (2009). Recurrence cancer stem cells--made by cell fusion? *Medical Hypotheses*, 73, 4, 542–57.
- Erenpreisa, J.; Cragg, M.S. (2010). MOS, aneuploidy and the ploidy cycle of cancer cells. *Oncogene*, [Epub ahead of print]
- Essers, M.A.; Trumpp, A. (2010). Targeting leukemic stem cells by breaking their dormancy. *Molecular Oncology*, [Epub ahead of print]
- Evers, P.; Lee, P.P.; DeMarco, J.; Agazaryan, N.; Sayre, J.W.; Selch, M.; Pajonk, F. (2010). Irradiation of the potential cancer stem cell niches in the adult brain improves progression-free survival of patients with malignant glioma. *BMC Cancer*, 21, 10, 384.
- Facchino, S.; Abdouh, M.; Chatoo, W.; Bernier, G. (2010). BMI1 confers radioresistance to normal and cancerous neural stem cells through recruitment of the DNA damage response machinery. *Journal of Neuroscience*, 30, 30, 10096–10111.
- Gasparini, P.; Bertolini, G.; Binda, M.; Magnifico, A.; Albano, L.; Tortoreto, M.; Pratesi, G.; Facchinetti, F.; Abolafio, G.; Roz, L.; Tagliabue, E.; Daidone, M.G.; Sozzi, G. (2010). Molecular cytogenetic characterization of stem-like cancer cells isolated from established cell lines. *Cancer Letters*, 296, 2, 206–215.
- Golding, S.E.; Rosenberg, E.; Valerie, N.; Hussaini, I.; Frigerio, M.; Cockcroft, X.F.; Chong, W.Y.; Hummersone, M.; Rigoreau, L.; Menear, K.A.; O'Connor, M.J.; Povirk, L.F.; van Meter, T.; Valerie, K. (2009). Improved ATM kinase inhibitor KU-60019 radiosensitizes glioma cells, compromises insulin, AKT and ERK prosurvival signaling, and inhibits migration and invasion. *Molecular Cancer Therapeutics*, 8, 10, 2894–2902.
- Guilbaud, N.F.; Gas, N.; Dupont, M.A.; Valette, A. (1990). Effects of differentiation-inducing agents on maturation of human MCF-7 breast cancer cells. *Journal of Cell Physiology*, 145, 1, 162–172.
- Gupta, P.B.; Onder, T.T.; Jiang, G.; Tao, K.; Kuperwasser, C.; Weinberg, R.A.; Lander, E.S. (2009). Identification of selective inhibitors of cancer stem cells by high-throughput screening. *Cell*, 138, 4, 645–659.
- Hamburger, A.W.; Salmon, S.E. (1977). Primary bioassay of human tumor stem cells. *Science* 197, 4302, 461–463.
- Harper, L.J.; Costea, D.E.; Gammon, L.; Fazil, B.; Biddle, A.; Mackenzie, I.C. (2010). Normal and malignant epithelial cells with stem-like properties have an extended G2 cell cycle phase that is associated with apoptotic resistance. *BMC Cancer*, 28, 10, 166.
- Hirschmann-Jax, C.; Foster, A.E.; Wulf, G.G.; Nuchtern, J.G.; Jax, T.W.; Gobel, U.; Goodell, M.A.; Brenner, M.K. (2004). A distinct "side population" of cells with high drug efflux capacity in human tumor cells. *Proceedings of the National Academy of Sciences USA*, 101, 39, 14228–14233.

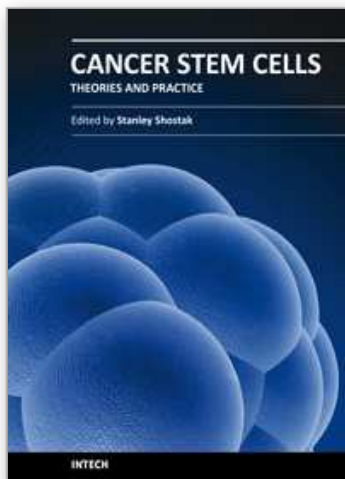
- Hochedlinger, K.; Blelloch, R.; Brennan, C.; Yamada, Y.; Kim, M.; Chin, L.; Jaenisch, R. (2004). Reprogramming of a melanoma genome by nuclear transplantation. *Genes & Development*, 18, 15, 1875-1885.
- Hong, D.; Gupta, R.; Ancliff, P.; Atzberger, A.; Brown, J.; Soneji, S.; Green, J.; Colman, S.; Piacibello, W.; Buckle, V.; Tsuzuki, S.; Greaves, M.; Enver, T. (2008). Initiating and cancer-propagating cells in TEL-AML1-associated childhood leukemia. *Science*, 319, 5861, 336-339.
- Ishimoto, T.; Oshima, H.; Oshima, M.; Kai, K.; Torii, R.; Masuko, T.; Baba, H.; Saya, H.; Nagano, O. (2010). CD44⁺ slow-cycling tumor cell expansion is triggered by cooperative actions of Wnt and prostaglandin E2 in gastric tumorigenesis. *Cancer Science*, 101, 3, 673-678.
- Jin, F.; Zhao, L.; Guo, Y.J.; Zhao, W.J.; Zhang, H.; Wang, H.T.; Shao, T.; Zhang, S.L.; Wei, Y.J.; Feng, J.; Jiang, X.B.; Zhao, H.Y. (2010). Influence of etoposide on anti-apoptotic and multidrug resistance-associated protein genes in CD133 positive U251 glioblastoma stem-like cells. *Brain Research*, 1336, 103-111.
- Kai, K.; Nagano, O.; Sugihara, E.; Arima, Y.; Sampetrean, O.; Ishimoto, T.; Nakanishi, M.; Ueno, N.T.; Iwase, H.; Saya, H. (2009). Maintenance of HCT116 colon cancer cell line conforms to a stochastic model but not a cancer stem cell model. *Cancer Science*, 100, 12, 2275-2282.
- Kelly, P.N.; Dakic, A.; Adams, J.M.; Nutt, S.L.; Strasser, A. (2007). Tumor growth need not be driven by rare cancer stem cells. *Science*, 317, 5836, 337.
- Kobayashi, H.; Man, S.; Graham, C.H.; Kapitan, S.J.; Teicher, B.A.; Kerbel, R.S. (1993). Acquired multicellular-mediated resistance to alkylating agents in cancer. *Proceedings of the National Academy of Sciences USA*, 90, 8, 3294-3298.
- Kulesa, P.M.; Kasemeier, J.C.; Teddy, J.M.; Margaryan, N.V.; Seftor, E.A.; Seftor, R.E.; Hendrix, M.J. (2006). Reprogramming metastatic melanoma cells to assume a neural crest-like phenotype in an embryonic microenvironment. *Proceedings of the National Academy of Sciences USA*, 103, 10, 3752-3757.
- Lee, J.; Kotliarova, S.; Kotliarov, Y.; Li, A.; Su, Q.; Donin, N.M.; Pastorino, S.; Purow, B.W.; Christopher, N.; Zhang, W.; Park, J.K.; Fine, H.A. (2006). Tumor stem cells derived from glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. *Cancer Cell*, 9, 5, 391-403.
- Levina, V.; Marrangoni, A.M.; DeMarco, R.; Gorelik, E.; Lokshin, A.E. (2008). Drug-selected human lung cancer stem cells: cytokine network, tumorigenic and metastatic properties. *PLoS One*, 3, 8, e3077.
- Levina, V.; Marrangoni, A.; Wang, T.; Parikh, S.; Su, Y.; Herberman, R.; Lokshin, A.; Gorelik, E. (2010). Elimination of human lung cancer stem cells through targeting of the stem cell factor-c-kit autocrine signaling loop. *Cancer Research*, 70, 1, 338-346.
- Li, H.; Chen, X.; Calhoun-Davis, T.; Claypool, K.; Tang, D.G. (2008). PC3 human prostate carcinoma cell holoclones contain self-renewing tumor-initiating cells. *Cancer Research*, 68, 6, 1820-1825.
- Li, H.; Collado, M.; Villasante, A.; Strati, K.; Ortega, S.; Cañamero, M.; Blasco, M.A.; Serrano, M. (2009). The Ink4/Arf locus is a barrier for iPS cell reprogramming. *Nature*, 460, 7259, 1136-1139.

- Lin, S.Y.; Elledge, S.J. (2003). Multiple tumor suppressor pathways negatively regulate telomerase. *Cell*, 113, 7, 881-889.
- Locke, M.; Heywood, M.; Fawell, S.; Mackenzie, I.C. (2005) Retention of intrinsic stem cell hierarchies in carcinoma-derived cell lines. *Cancer Research*, 65, 19, 8944-8950.
- Mani, S.A.; Guo, W.; Liao, M.J.; Eaton, E.N.; Ayyanan, A.; Zhou, A.Y.; Brooks, M.; Reinhard, F.; Zhang, C.C.; Shipitsin, M.; Campbell, L.L.; Polyak, K.; Briskin, C.; Yang, J.; Weinberg, R.A. (2008). The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell*, 133, 4, 704-715.
- Marian, C.O.; Cho, S.K.; Hatanpaa, K.J.; Wright, W.E.; Maher, E.; Madden, C.; Mickey, B.; Yang, X.-L.; Shay, J.W.; Bachoo, R. (2010). The telomerase antagonist, imetelstat, efficiently targets glioblastoma tumor-initiating cells leading to decreased proliferation and tumor growth. *Clinical Cancer Research*, 16, 1, 154-163.
- Marian, C.O.; Wright, W.E.; Shay, J.W. (2010). The effect of telomerase inhibition on prostate tumor-initiating cells. *International Journal of Cancer*, 127, 2, 321-331.
- Marsh, W.; Sicheri, D.; Center, M.S. (1986). Isolation and characterization of adriamycin-resistant HL-60 cells which are not defective in the initial intracellular accumulation of drug. *Cancer Research*, 46, 8, 4053-4057.
- Meng, X.; Li, M.; Wang, X.; Wang, Y.; Ma, D. (2009). Both CD133+ and CD133- subpopulations of A549 and H446 cells contain cancer-initiating cells. *Cancer Science*, 100, 6, 1040-1046.
- Meng, X.; Wang, X.; Wang, Y. (2009). More than 45% of A549 and H446 cells are cancer initiating cells: evidence from cloning and tumorigenic analyses. *Oncology Reports*, 21, 4, 995-1000.
- McCord, A.M.; Jamal, M.; Williams, E.S.; Camphausen, K.; Tofilon, P.J. (2009). CD133+ glioblastoma stem-like cells are radiosensitive with a defective DNA damage response compared with established cell lines. *Clinical Cancer Research*, 15, 16, 5145-5153.
- McCord, A.M.; Jamal, M.; Shankavaram, U.T.; Lang, F.F.; Camphausen, K.; Tofilon, P.J. (2009). Physiologic oxygen concentration enhances the stem-like properties of CD133+ human glioblastoma cells in vitro. *Molecular Cancer Research*, 7, 4, 489-497.
- Molina, J.R.; Hayashi, Y.; Stephens, C.; Georgescu, M.M. (2010). Invasive glioblastoma cells acquire stemness and increased Akt activation. *Neoplasia*, 12, 6, 453-463.
- Morel, A.P.; Lièvre, M.; Thomas, C.; Hinkal, G.; Ansieau, S.; Puisieux, A. (2008). Generation of breast cancer stem cells through epithelial-mesenchymal transition. *PLoS One*, 3, 8, e2888.
- Mueller, M.T.; Hermann, P.C.; Witthauer, J.; Rubio-Viqueira, B.; Leicht, S.F.; Huber, S.; Ellwart, J.W.; Mustafa, M.; Bartenstein, P.; D'Haese, J.G.; Schoenberg, M.H.; Berger, F.; Jauch, K.W.; Hidalgo, M.; Heeschen, C. (2009). Combined targeted treatment to eliminate tumorigenic cancer stem cells in human pancreatic cancer. *Gastroenterology*, 137, 3, 1102-1113.
- Mumcuoglu, M.; Bagislar, S.; Yuzugullu, H.; Alotaibi, H.; Senturk, S.; Telkoparan, P.; Gur-Dedeoglu, B.; Cingoz, B.; Bozkurt, B.; Tazebay, U.H.; Yulug, I.G.; Akcali, K.C.; Ozturk, M. (2010). The ability to generate senescent progeny as a mechanism underlying breast cancer cell heterogeneity. *PLoS One*, 5, 6, e11288.

- Nakai, E.; Park, K.; Yawata, T.; Chihara, T.; Kumazawa, A.; Nakabayashi, H.; Shimizu, K. (2009). Enhanced MDR1 expression and chemoresistance of cancer stem cells derived from glioblastoma. *Cancer Investigations*, 27, 9, 901-908.
- Ozturk, N.; Erdal, E.; Mumcuoglu, M.; Akcali, K.C.; Yalcin, O.; Senturk, S.; Arslan-Ergul, A.; Gur, B.; Yulug, I.; Cetin-Atalay, R.; Yakicier, C.; Yagci, T.; Tez, M.; Ozturk, M. (2006). Reprogramming of replicative senescence in hepatocellular carcinoma-derived cells. *Proceedings of the National Academy of Sciences USA*, 103, 7, 2178-2183.
- Quintana, E.; Shackleton, M.; Sabel, M.S.; Fullen, D.R.; Johnson, T.M.; Morrison, S.J. (2008). Efficient tumour formation by single human melanoma cells. *Nature*, 456, 7222, 593-598.
- Pardal, R.; Clarke, M.F.; Morrison, S.J. (2003). Applying the principles of stem-cell biology to cancer. *Nature Reviews on Cancer*, 3, 12, 895-902.
- Patrawala, L.; Calhoun, T.; Schneider-Broussard, R.; Zhou, J.; Claypool, K.; Tang, D.G. (2005). Side population is enriched in tumorigenic, stem-like cancer cells, whereas ABCG2+ and ABCG2- cancer cells are similarly tumorigenic. *Cancer Research*, 65, 14, 6207-6219.
- Pralong, D.; Trounson, A.O.; Verma, P.J. (2006). Cell fusion for reprogramming pluripotency: toward elimination of the pluripotent genome. *Stem Cell Reviews*, 2, 4, 331-340.
- Raaijmakers, M.H.; Mukherjee, S.; Guo, S.; Zhang, S.; Kobayashi, T.; Schoonmaker, J.A.; Ebert, B.L.; Al-Shahrour, F.; Hasserjian, R.P.; Scadden, E.O.; Aung, Z.; Matza, M.; Merckenschlager, M.; Lin, C.; Rommens, J.M.; Scadden, D.T. (2010). Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia. *Nature*, 464, 7290, 852-857.
- Reya, T.; Morrison, S.J.; Clarke, M.F.; Weissman, I.L. (2001). Stem cells, cancer, and cancer stem cells. *Nature*, 414, 6859, 105-111.
- Riccioni, R.; Dupuis, M.L.; Bernabei, M.; Petrucci, E.; Pasquini, L.; Mariani, G.; Cianfriglia, M.; Testa, U. (2010). The cancer stem cell selective inhibitor salinomycin is a p-glycoprotein inhibitor. *Blood Cells, Molecules and Diseases*, 45, 1, 86-92.
- Rizvi, A.Z.; Swain, J.R.; Davies, P.S.; Bailey, A.S.; Decker, A.D.; Willenbring, H.; Grompe, M.; Fleming, W.H.; Wong, M.H. (2006). Bone marrow-derived cells fuse with normal and transformed intestinal stem cells. *Proceedings of the National Academy of Sciences USA*, 103, 16, 6321-6315.
- Ron-Bigger, S.; Bar-Nur, O.; Isaac, S.; Bocker, M.; Lyko, F.; Eden, A. (2010). Aberrant epigenetic silencing of tumor suppressor genes is reversed by direct reprogramming. *Stem Cells*, [Epub ahead of print]
- Roninson, I.B. (2003). Tumor cell senescence in cancer treatment. *Cancer Research*, 63, 11, 2705-2715.
- Ropolo, M.; Daga, A.; Griffero, F.; Foresta, M.; Casartelli, G.; Zunino, A.; Poggi, A.; Cappelli, E.; Zona, G.; Spaziante, R.; Corte, G.; Frosina G. (2009). Comparative analysis of DNA repair in stem and nonstem glioma cell cultures. *Molecular Cancer Research*, 7, 3, 383-392.
- Sabisz, M.; Skladanowski, A. (2009). Cancer stem cells and escape from drug-induced premature senescence in human lung tumor cells: implications for drug resistance and in vitro drug screening models. *Cell Cycle*, 8, 19, 3208-3217.

- Saito, Y.; Kitamura, H.; Hijikata, A.; Tomizawa-Murasawa, M.; Tanaka, S.; Takagi, S.; Uchida, N.; Suzuki, N.; Sone, A.; Najima, Y.; Ozawa, H.; Wake, A.; Taniguchi, S.; Shultz, L.D.; Ohara, O.; Ishikawa, F. (2010). Identification of therapeutic targets for quiescent, chemotherapy-resistant human leukemia stem cells. *Science Translational Medicine*, 2, 17, 17ra9.
- Salmina, K.; Jankevics, E.; Huna, A.; Perminov, D.; Radovica, I.; Klymenko, T.; Ivanov, A.; Jascenko, E.; Scherthan, H.; Cragg, M.; Erenpreisa, J. (2010). Up-regulation of the embryonic self-renewal network through reversible polyploidy in irradiated p53-mutant tumour cells. *Experimental Cell Research*, 316, 13, 2099-2112.
- Santisteban, M.; Reiman, J.M.; Asiedu, M.K.; Behrens, M.D.; Nassar, A.; Kalli, K.R.; Haluska, P.; Ingle, J.N.; Hartmann, L.C.; Manjili, M.H.; Radisky, D.C.; Ferrone, S.; Knutson, K.L. (2009). Immune-induced epithelial to mesenchymal transition in vivo generates breast cancer stem cells. *Cancer Research*, 69, 7, 2887-2895.
- Setoguchi, T.; Taga, T.; Kondo, T. (2004). Cancer stem cells persist in many cancer cell lines. *Cell Cycle*, 3, 4, 414-15.
- Shen, G.; Shen, F.; Shi, Z.; Liu, W.; Hu, W.; Zheng, X.; Wen, L.; Yang, X. (2008). Identification of cancer stem-like cells in the C6 glioma cell line and the limitation of current identification methods. *In Vitro Cellular and Developmental Biology - Animal*, 44, 7, 280-9.
- Souza, G.R.; Molina, J.R.; Raphael, R.M.; Ozawa, M.G.; Stark, D.J.; Levin, C.S.; Bronk, L.F.; Ananta, J.S.; Mandelin, J.; Georgescu, M.M.; Bankson, J.A.; Gelovani, J.G.; Killian, T.C.; Arap, W.; Pasqualini, R. (2010). Three-dimensional tissue culture based on magnetic cell levitation. *Nature Nanotechnology*, 5, 4, 291-296.
- Skladanowski, A.; Bozko, P.; Sabisz, M. (2009). DNA structure and integrity checkpoints during the cell cycle and their role in drug targeting and sensitivity of tumor cells to anticancer treatment. *Chemical Reviews*, 109, 7, 2951-2973.
- Soddu, S.; Blandino, G.; Citro, G.; Scardigli, R.; Piaggio, G.; Ferber, A.; Calabretta, B.; Sacchi, A. (1994). Wild-type p53 gene expression induces granulocytic differentiation of HL-60 cells. *Blood*, 83, 8, 2230-2237.
- Sussman, R.T.; Ricci, M.S.; Hart, L.S.; Sun, S.Y.; El-Deiry, W.S. (2007) Chemotherapy-resistant side-population of colon cancer cells has a higher sensitivity to TRAIL than the non-SP, a higher expression of c-Myc and TRAIL-receptor DR4. *Cancer Biology & Therapy*, 6, 9, 1490-1495.
- Tilkorn, D.J.; Lokmic, Z.; Chaffer, C.L.; Mitchell, G.M.; Morrison, W.A.; Thompson, E.W. (2010). Disparate companions: tissue engineering meets cancer research. *Cells Tissues & Organs*, 192, 3, 141-157.
- Thomas, X.; Cannas, G. (2010). Leukemia stem cells and new strategies to overcome resistance to therapy. *Current Stem Cell Research and Therapy*, 5, 3, 277-286.
- Vakifahmetoglu, H.; Olsson, M.; Zhivotovsky, B. (2008). Death through a tragedy: mitotic catastrophe. *Cell Death & Differentiation*, 15, 7, 1153-1162.
- Vermeulen, L.; De Sousa, E.; Melo, F.; van der Heijden, M.; Cameron, K.; de Jong, J.H.; Borovski, T.; Tuynman, J.B.; Todaro, M.; Merz, C.; Rodermond, H.; Sprick, M.R.; Kemper, K.; Richel, D.J.; Stassi, G.; Medema, J.P. (2010). Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nature Cell Biology*, 12, 5, 468-476.

- Verschueren, K.; Remacle, J.E.; Collart, C.; Kraft, H.; Baker, B.S.; Tylzanowski, P.; Nelles, L.; Wuytens, G.; Su, M.T.; Bodmer, R.; Smith, J.C.; Huylebroeck, D. (1999). SIP1, a novel zinc finger/homeodomain repressor, interacts with Smad proteins and binds to 5'-CACCT sequences in candidate target genes. *The Journal of Biological Chemistry*, 274, 29, 20489-2098.
- Wang, F.; Weaver, V.M.; Petersen, O.W.; Larabell, C.A.; Dedhar, S.; Briand, P.; Lupu, R.; Bissell, M.J. (1998). Reciprocal interactions between beta1-integrin and epidermal growth factor receptor in three-dimensional basement membrane breast cultures: a different perspective in epithelial biology. *Proceedings of the National Academy of Sciences USA*, 95, 25, 14821-14826.
- Wang, J.; Xie, L.Y.; Allan, S.; Beach, D.; Hannon, G.J. (1998). Myc activates telomerase. *Genes & Development*, 12, 12, 1769-1774.
- Wang, X.Q.; Ongkeko, W.M.; Chen, L.; Yang, Z.F.; Lu, P.; Chen, K.K.; Lopez, J.P.; Poon, R.T.; Fan, S.T. (2010). Octamer 4 (Oct4) mediates chemotherapeutic drug resistance in liver cancer cells through a potential Oct4-AKT-ATP-binding cassette G2 pathway. *Hepatology*, 52, 2, 528-539.
- Wang, Y.K.I.; Zhu, Y.L.; Qiu, F.M.; Zhang, T.; Chen, Z.G.; Zheng, S.; Huang, J. (2010). Activation of Akt and MAPK pathways enhances the tumorigenicity of CD133+ primary colon cancer cells. *Carcinogenesis*, 31, 8, 1376-1380.
- Wasserman, L.; Nordenberg, J.; Beery, E.; Deutsch, A.A.; Novogrodsky, A. (1987). Differential effects of sodium butyrate and dimethylsulfoxide on gamma-glutamyl transpeptidase and alkaline phosphatase activities in MCF-7 breast cancer cells. *Experimental Cell Biology*, 55, 4, 188-193.
- Wicha, M.S.; Liu, S.; Dontu, G. (2006). Cancer stem cells: an old idea--a paradigm shift. *Cancer Research*, 66, 4, 1883-1890.
- Yamamoto, A.; Shofuda, T.; Islam, M.O.; Nakamura, Y.; Yamasaki, M.; Okano, H.; Kanemura, Y. (2009). ABCB1 is predominantly expressed in human fetal neural stem/progenitor cells at an early development stage. *J Neuroscience Research*, 87, 12, 2615-2623.
- Yamanaka, S.; Blau, H.M. (2010). Nuclear reprogramming to a pluripotent state by three approaches. *Nature*, 465, 7299, 704-712.
- Yeung, T.M.; Gandhi, S.C.; Wilding, J.L.; Muschel, R.; Bodmer, W.F. (2010). Cancer stem cells from colorectal cancer-derived cell lines. *Proceedings of the National Academy of Sciences USA*, 107, 8, 3722-3727.
- Ying, Q.-L.; Wray, J.; Nichols, J.; Battle-Morera, L.; Doble, B.; Woodgett J.; Cohen P.; Smith A. (2008). The ground state of embryonic stem cell self-renewal. *Nature*, 453, 7194, 519-523.
- Qian, Y.; Drewe, J.A.; Shi, J.; McClurg, M.; Li, W.; Campbell, T.N. Tumorase™: *In vivo* efficacy data for novel localized proteinase treatment applicable to solid tumors of varied tissue origins, *Proceedings of the American Association for Cancer Research*, Washington DC, April, 2010, Cadmus Publishing, Richmond VA.
- Zhang, M.; Atkinson, R.L.; Rosen, J.M. (2010). Selective targeting of radiation-resistant tumor-initiating cells. *Proceedings of the National Academy of Sciences USA*, 107, 8, 3522-3527.
- Zheng, X.; Shen, G.; Yang, X.; Liu, W. (2007). Most C6 cells are cancer stem cells: evidence from clonal and population analyses. *Cancer Research*, 67, 8, 3691-3697.



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